

**KW 38:**

a) Plasmid-isolation of the backbone pmz333 (insert included) of two overnight cultures

- midiprep according to Qiagen-protocol
- results: pmz333.1 with 220,3ng/ul and pmz333.2 with 491,3 ng/ul
- test digest with PuvII-HF: desired DNA lengths were seen
- complete digest of pmz333.2 (because of higher concentrations): 20 ul plasmid with each 2,5 ul XbaI and NotI-Hf for 3 hours
- gel-extraction with Qiagen Kit
  - ➔ "pmz.333.2 cut backbone"

b) construct 3 &4:

G-block construct 3: LOV-GFP-TOM5

G-block construct 4: PDZ-mCherry-Bax184e

1. adding restriction sites NotI and XbaI to the G-blocks:

- Phusion-PCR with the following recipe:
  - 1 ul plasmid construct 3 with Primer RI0016, RI0036
  - 1 ul plasmid construct 4 with Primer RI0017, RI0038
- thermocycler options: 98(30s), 20\* [98(10s), 64 (30s), 72(1:10s)], 74 (5min)
- DPN1 digest with the PCR-products
- successful PCR according to test gel: LOV (~1,4kb), PDZ (2kb)